

TES

Site:	Syntex-Verona
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Reports	

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**Transport, Fate, and Bioavailability of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Aquatic Environments**

**BACKGROUND:** Few environmental contaminants have received the attention of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This compound has been the focal point of numerous state and federal agencies in the U.S., Canada, and other countries. It has recently come to the forefront of environmental problems in Missouri. The cost of the dioxin investigations in Missouri may reach unprecedented proportions. The U.S. Environmental Protection Agency has spent an estimated 33 million dollars since 1980 on sampling and analysis. An additional 30 million dollars have been appropriated for the purchase of contaminated sites, and an additional estimated 100-500 million dollars have been projected as needed to fund cleanup alternatives. The Missouri Department of Natural Resources has expended over 5 million dollars of state money on similar investigations during the same time period.

Hazardous waste sites with high levels of TCDD contamination that threaten ground and surface water resources are of particular concern. The Spring River was the first area where environmental contamination with TCDD was reported in Missouri. The manufacture of the herbicide 2,4,5-T and the disinfectant hexachlorophene in the early 1970's at an industrial plant in Verona produced several waste streams that contained TCDD. The deposition of this waste material near the Spring River at Verona and in other nearby locations in southwest Missouri has been the subject of exhaustive research by the U.S. Environmental Protection Agency since 1978. Eight potential disposal sites have been confirmed near the industrial complex presently owned by Syntex Agribusiness. Three of these sites are located on the flood plain and are possible sources of TCDD contamination in the Spring River.



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SUPERFUND RECORDS

TCDD was first reported in soil and sediment samples taken from a spring and pasture near the Spring River two miles downstream from the Syntex plant in July of 1981. Subsequent investigations by the U.S. Environmental Protection Agency and the Missouri Department of Natural Resources have identified over 40 additional sites contaminated with TCDD including the entire town of Times Beach, Missouri. TCDD was first found in Spring River fish in December 1981. Whole-body concentrations were 52, 39, 6.2, 2.5, and .8 pptr at .27, 2.75, 33, 43, and 66 miles downstream respectively, of the Syntex plant. Concentrations in fillets were 18, 15, 17, 6, and 1.4 pptr at .27, 1.44, 2.75, 8, 33, and 43 miles downstream, respectively. Other aquatic organisms have subsequently been found to contain TCDD in the Spring River(1). Benthic macroinvertebrates, crayfish and freshwater mussels have reported concentrations between 3 and 12 pptr. The U.S. Food and Drug Administration advisory levels for edible fish tissue are between 25 and 50 pptr. Due to these elevated concentrations, a health advisory was issued for people consuming fish from the Spring River.

The 2,3,7,8-TCDD isomer has been shown to be extremely toxic to a variety of mammals (2,3). The pathological response includes edema formation (4,5), effects on the immune system (6,7) and liver damage (8,9). TCDD has been reported to be teratogenic in mammals (10) and is reported to be one of the most toxic compounds known. TCDD is also extremely toxic to aquatic organisms. Helder (11) demonstrated that exposure of eyed pike eggs to TCDD concentrations ranging from .1 to 10 pptr for 4 days resulted in decreased growth and survival. All fish exposed to the highest TCDD concentration died within 21 days. Juvenile rainbow trout exposed to concentrations of 10 and 100 pptr for 4 days, exhibited reduced growth and developed limited edematous changes (12). Mortality occurred at the highest concentrations within 27

days. Mehrle et. al. (13) reported TCDD induced significant effects on survival, growth, and behavior of rainbow trout at a concentration less than 38 pg/l in 28-day exposures. Johnson et al. (15) reported a variety of pathological lesions in carp exposed to TCDD at 100 ppq for 71 days. TCDD is also bioaccumulated in aquatic organisms. Bio-concentration factors ranging from  $2-2.6 \times 10^4$  have been reported for snails, fish and daphnids exposed for 3-6 days in a model ecosystem (14). The actual impacts of TCDD on riverine life of the Spring River and the potential for bioaccumulation are unknown.

Knowledge of the transport, distribution and fate of TCDD in aquatic ecosystems is of paramount importance in delineating the environmental impact and aiding in selecting potential mitigative actions at the Verona, Missouri plant site. The mechanism of transport of TCDD from contaminated sites to the river is unknown. Two hypotheses are considered; 1) TCDD bound to soil particles near the surface is transported by erosion into the river, 2) concentrations of TCDD below existing detection limits are being solubilized and carried through alluvial gravel to the river. Knowledge of the transport mechanisms will be useful in formulating successful cleanup measures.

The proposed study will involve assessment of the bioavailability of dioxin to fish in the Spring River at Verona, Missouri. The study is divided into five phases which are briefly outlined below.

- A. Laboratory bioaccumulation: This phase will consist of exposing fingerling fish to ground water potentially contaminated with TCDD and determining if uptake occurs.
- B. Surface runoff: The second phase will consist of collecting a series of water samples during storm runoff and analyzing the dissolved and suspended solids fractions for TCDD.

- C. Caged fish: Fish will be confined within wire enclosures in the Spring River to determine if fish accumulate TCDD under natural conditions.
- D. Bioaccumulation from food: Fish exposed in the laboratory bioaccumulation phase (A) and in the caged fish phase (C) will be fed a diet which contains TCDD. The results of these feeding experiments in conjunction with the water exposure experiments will help identify the relative contribution of food and water to the total body burden of TCDD.
- E. Histopathological studies: Fish exposed in the laboratory bioaccumulation and caged fish phase will be examined for histopathological lesions at the end of the 60-day exposure period.

The above studies will provide information on the following:

1. Presence of TCDD in water and suspended solids
2. Analytical capability of detecting low levels (<pg/L) TCDD in water and suspended particles
3. An estimate of free versus-bound TCDD
4. Ground water levels of TCDD
5. Bioconcentration of TCDD by fish from ground water and stream water
6. Interpretation of the relationship between the concentration of TCDD in stream sediments and biota from the Spring River site.
7. Relative contribution of food and water to total body burden of TCDD.
8. Histopathological lesions associated with contaminant exposure.

#### STUDY PROTOCOL

**OBJECTIVE:** To determine the transport, fate and bioavailability of 2,3,7,8-TCDD in the aquatic environment.

**PURPOSE:** This study is intended to provide additional information about the transport of TCDD to the Spring River and effects on aquatic organisms. Knowledge of these processes may be important in developing strategies to reduce and prevent further contamination of aquatic habitat at other TCDD

contaminated sites in Missouri. This approach will also be valuable in addressing the problems of the transport, fate and bioavailability of other aquatic contaminants in Missouri. A secondary goal is to compare the uptake rates of TCDD for exposure to water and exposure to food which contains TCDD. If it is possible to identify which component is the major contributor, it would provide further information which could be useful in reducing costly mitigation efforts to the minimum effective level.

Accumulation of 2,3,7,8-TCDD in Spring River fish is hypothesized to be controlled by sediment bound TCDD eroding into the river or by solubilized TCDD dissolved in ground water entering the river. Ground water transport may be mediated by the presence of naturally occurring organic substances present in the water or by the presence of organic solvents which have been identified in the ground water. TCDD has not been found in samples of ground water taken from the site. The predicted water concentration of TCDD based on the soil water partition coefficient is below the present level of detection for this compound in water. A biological monitoring system is proposed to be used to indicate the presence or absence of TCDD in ground water where current analytical methodology is insufficient to detect water concentrations. The monitoring system will expose fish to ground water for an extended period of time allowing bioaccumulation of persistent compounds. Fish will be analyzed at the end of the exposure period to determine if TCDD has accumulated. If TCDD is found in fish, the estimated water concentration can be calculated from the published bioconcentration factors for TCDD. If bioaccumulation does not occur, ground water transport can be eliminated as a mode of TCDD transport at this site.

Surface erosion of contaminated soil may play a role in the transport of TCDD to the Spring River. Other streams in Missouri have been found to

contain TCDD in sediment and biota following placement of contaminated soil in the watershed. Water samples will be collected from drainages which traverse the Syntex plant site during storm events and analyzed for TCDD.

Fish from the Spring River analyzed for TCDD in earlier studies have spent time in non-contaminated portions of the stream and therefore the residue levels detected may not necessarily reflect the potential for higher residues in less mobile species. To ascertain the current bioavailability of TCDD residues and their bioconcentration potential in the Spring River, a "caged" fish study will be conducted. The residue-free fish will be placed in the Spring River, near the chemical plant, for a period of 30-60 days. At the end of the exposure period, the fish will be analyzed for TCDD residues. This study will provide data on the bioavailability of TCDD (river water with suspended particulates) to fish in a natural ecosystem and indicate the potential for food chain problems in wildlife (particularly birds) feeding on the fish. Bottom sediments have been collected on an annual basis since 1983 beginning above the plant site and downstream for approximately 10 river miles. These samples have been analyzed for TCDD residues. River sediment has also been characterized as to texture, organic content and mineralogical composition. This information will be useful in interpreting the caged fish studies.

TCDD is so insoluble in water that concentrations in fish may be controlled by partitioning of the compound from organic components of the water and suspended solids in the water to the lipid phase of the fish tissue. Under these conditions ingestion of food organisms may be an important route of TCDD uptake. To determine the contribution of food, an experiment will be conducted using a natural food source which has been environmentally contaminated with TCDD. The experiment will be designed to determine the

individual contributions of TCDD in water and in food to the total body burden in fish. Past residue analysis for TCDD in biota has shown similar concentrations of TCDD in fish and in benthic invertebrates available for consumption. The contaminated diet is intended to represent the concentration of TCDD in fish food organisms found in the Spring River which would normally be consumed by resident fish. If fish accumulate a large portion of TCDD from food, but not from exposure water this would provide evidence that TCDD uptake is the result of ingestion of contaminated food rather than water.

#### Experimental Design

The bioaccumulation study will involve a 60-day exposure period with juvenile rainbow trout Salmo gairdneri followed by a 120-day depuration period. Rainbow trout were selected as the test species because they have been used in similar studies with TCDD where bioconcentration factors have been reported (16). Four treatments will be used with 2 replicates per treatment. Two treatments will be supplied with uncontaminated ground water from an upgradient monitoring well and two treatments will be supplied with ground water from monitoring well 8 located on the Syntex property in Verona. One treatment from each group will be fed an uncontaminated diet and one will be fed a diet environmentally contaminated with TCDD.

Two additional treatments had been proposed which were to be dosed with .020 ng/l of TCDD. This concentration was chosen because it was half the no-effect concentration reported for TCDD in rainbow trout (13).

Approximately <sup>500</sup>50 nanograms of TCDD would be required for these control treatments. However, production of any additional TCDD contaminated material could pose an ethical problem and this portion of the study has been recommended to be eliminated. The exposure matrix is outlined in Table 1.

Table 1.

Treatment	Food	Water
1	Standard diet	Diluted well effluent
2	TCDD contaminated diet (app. 20 ppt.)	Diluted well effluent
3	Standard diet	Uncontaminated upgradient ground water
4	TCDD contaminated diet	Uncontaminated upgradient ground water
5*	Standard diet	Diluted well effluent dosed with 20 ng/l TCDD
6*	TCDD contaminated diet	

TCDD will be measured in fish from each treatment at 0, 15, 30, 45 and 60 days by GC/MS. Fish will be removed from the exposure chambers at 60 days and placed in another facility for depuration. If TCDD accumulation occurs, additional fish will be analyzed at 90 and 120 days to determine the elimination rate. Water samples will be taken at 0, 30 and 60 days from treatment 1 (diluted well effluent) and treatment 3 (uncontaminated upgradient ground water) and analyzed for TCDD. Alkalinity, hardness, and total organic carbon will be determined weekly for all treatments. Dissolved oxygen, temperature, pH, conductivity, and ammonia will be analyzed daily.

The caged fish study will involve a 60-day exposure with juvenile rainbow trout. Cages will be placed immediately downstream of the Syntex plant site in the Spring River. Two treatments will be used with two replicates per treatment. One treatment will be fed a standard diet and the other will be fed a diet environmentally contaminated with TCDD. TCDD will be measured in fish from each treatment at 0, 15, 30, 45, and 60 days by GC/MS. Remaining fish will be removed from the enclosure at 60 days and placed in another facility for depuration. If TCDD accumulation occurs, the fish which have

\* Recommended to be eliminated



been placed in clean water for 60 days will be analyzed at 90 and 120 days to determine the elimination rate. Water samples will be taken at 0, 30 and 60 days and analyzed for TCDD. Alkalinity hardness, and total organic carbon will be determined weekly. Dissolved oxygen, temperature, pH, conductivity and ammonia will be analyzed daily.

The histopathological study will require ten fish from each replicate of the laboratory and caged fish studies. They will be removed following 60 days of exposure, preserved in a suitable fixative, sectioned, stained and examined by light microscopy. An attempt will be made to obtain rainbow trout of the same size and age which have been exposed to TCDD under experimental conditions from other ongoing studies to use for comparative purposes. Additional examination may include preparations of certain tissues for evaluation with electron microscopy.

#### Exposure Facility

Bioassay tests will be performed within a 8' x 24' trailer mounted laboratory. All fish exposures will employ covered tanks under negative pressure vented to the outside. Twelve 40-liter glass aquaria will be held in a temperature controlled water bath which will be covered with a folding plexiglass top for access and observation. The flow-through bioassay exposure unit will produce approximately 2,000 liters of water per day for discharge. Approximately 10 liters of this total will be drawn from well #8 with a micro-syringe pump and diluted to less than 1% by volume with uncontaminated upgradient well water. It is anticipated that this discharge water (treatments 1-4) will not require special treatment and will be continuously irrigated onto the ground through a short length of perforated plastic pipe. If treatment 5 and 6 are included, a clean-up system will be used to remove

TCDD from the discharge water. Carbon absorption beds have been shown to reduce TCDD concentration by greater than 99% from solutions which contain TCDD in the low nanogram per liter range.

#### Preparation of Fish Diets

The diets will be prepared from ground fish stabilized with a gelatin binder. The contaminated diet will consist of white suckers Catostomus commersoni which will be collected near Verona in the Spring River where TCDD has been previously documented in fish. The uncontaminated diet will be prepared similarly, but with white suckers collected from a river with no known contamination. Fish will be fed approximately 3% of their body weight per day. Approximately 4 kg of each diet will be required for the duration of the study. The concentration of TCDD in the diets will be measured by GC/MS.

#### Bioassay Conditions

Approximately 600 five-gram trout, 50 per each of 12 tanks will be required to obtain an adequate amount of fish tissue for analysis in the bioaccumulation study. Approximately 25 grams (5 fish) will be necessary for each GC/MS analysis. A total of 250 grams will be stocked for each 40 l tank. This would require 100 grams of fish per tank for the exposure portion and 150 grams for the depuration and histopathological analysis. The loading of fish in aquaria will be 6.2 g/l. Assuming a turnover rate of 2.5 times per day the loading will be 2.5 grams of fish per liter of water. About 2,000 l of dilution water per day will be required for the study. The dilution water will be hauled or piped from an uncontaminated upgradient well located approximately 1.2 km from the mobile lab.

The caged fish study will require 200 twenty-gram trout, 50 per each of the four enclosures. Eighty grams will be used for each analysis. This would allow approximately 320 grams for the exposure portion and the 280 grams for the depuration and histopathology portion of the test.

Chemical Analysis: Chemical analysis for TCDD will be by contract laboratory.

High resolution gas chromatography and mass spectrometry with the following detection limits will be required: fish tissue and sediment - less than 5 parts per trillion; water - less than .5 parts per trillion. U.S. EPA protocols will be followed where appropriate.

Personal Safety Protection: The three investigators involved in the study will have a minimum of 40 hours of personal protection training and will have health physicals before working on the site.

Safe working procedures will be outlined and followed strictly to minimize contact with potentially hazardous substances.

PRINCIPAL INVESTIGATORS: Ronald L. Crunkilton, Robert J. DiStefano and Cynthia S. Morris.

GEOGRAPHIC LOCATION: Spring River at Verona, MO.

DURATION OF STUDY: 1987-1989.

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Table 1.

Job Objectives	Time Frame
Equipment procurement & method development	June 1987 - December 1987
Ground water bioaccumulation study	January 1988 - June 1988
Surface runoff transport study	June 1988 - September 1988
Caged fish study	September 1988 - December 1988
Data analysis & report preparation	January 1989 - December 1989